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Aquaculture
Aquaculture 225 (2003) 67–82
www.elsevier.com/locate/aqua-online

Nutritional value of heat-treated soybean meal for channel catfish (*Ictalurus punctatus*)

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Abstract

A study was undertaken to evaluate the effect of heat treatment of defatted raw soybean meal (RSBM) on the growth performance, hematology, immune response and resistance of channel catfish to *Edwardsiella ictaluri* challenge. Six practical-type diets were formulated to be isocaloric (2.8 kcal DE/kg diet) and isonitrogenous (34% crude protein). A diet containing 45% of commercial soybean meal (CSBM) served as the control. RSBM, non-heat treated (RSBM0) and heated in an autoclave using the dry cycle at 130 °C and 22 psi for 5 (RSBM5), 10 (RSBM10), 20 (RSBM20), and 40 min (RSBM40), was used to isonitrogenously replace the CSBM in the control diet. Each diet was fed to juvenile catfish (4.98 g) in triplicate aquaria twice daily to apparent satiation for 10 weeks. Another batch of diets containing 1% of chromic oxide was used for measurement of apparent digestibility coefficients. Heating RSBM for 20 min or longer lowered trypsin inhibitor (TI) content and increased the apparent protein digestibility (APD). The protein dispersibility index (PDI) decreased with an increase in the duration of heat treatment. Fish fed CSBM and RSBM40 diets had similar weight gain, protein efficiency ratio and apparent protein utilization which were significantly higher than those of fish fed the other diets. Feed intake significantly increased when RSBM was heated for 20 min or longer. Fish fed the RSBM40 diet had significantly lower feed intake but higher feed efficiency than those fed the CSBM diet. Whole-body protein was highest for fish fed the RSBM40 diet but did not differ from that of fish fed the CSBM diet. Hepatosomatic and visceral indices (HSI and VI) of the groups fed CSBM and RSBM40 diets were significantly lower than those fed other diets. Total cell count, red blood cell count, hematocrit and hemoglobin were not significantly affected by dietary treatment. Plasma lysozyme activity and protein of fish fed the CSBM and RSBM40 diets were similar and significantly higher than those of fish fed the other diets. Cumulative mortality 14-day post challenge with *E. ictaluri* was significantly lower for fish fed the RSMB5 and RSBM10 diets but significantly increased when heating time was increased to 40 min. Macrophage chemotaxis in the presence of exoantigen and antibody titer against *E. ictaluri* was

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doi:10.1016/S0044-8486(03)00289-8

higher for the groups fed the RSBM containing diets than those fed the CSBM diet, although the differences were not always significant. Results of this study indicate that autoclaving the RSBM for 40 min lowered the TI and PDI, improved the nutritional value of RSBM and increased plasma lysozyme and protein. However, this level of heat treatment significantly decreased macrophage chemotaxis, antibody titer and resistance of fish to *E. ictaluri* challenge.

Keywords: Channel catfish; Soybean meal; Heat treatment; Growth performance; Immune response; Disease resistance

1. Introduction

At present, because of its nutritional value and palatability, fish meal still constitutes a substantial portion of fish feed formula. However, the scarcity and rising cost of fish meal has stimulated research on alternative protein sources, particularly plant proteins. Soybean meal (SBM) is the most commonly used plant protein in feed formulation for aquaculture species (Lim and Akiyama, 1991). Many studies have been carried out to examine the effects of the partial or total replacement of fish meal by SBM. Most results showed that incorporation of high levels of SBM in fish diets has frequently lead to reduced growth performance and feed utilization efficiency (Beckmann and Pfeffer, 1989; Lim and Dominy, 1989). Reasons for poor utilization of SBM could be attributed to a number of factors such as an improper balance of essential nutrients (amino acids, energy and minerals), lower digestibility, reduced diet palatability and the presence of anti-nutritional factors such as trypsin inhibitors (TI) and lectins (Tacon, 1994). Previous studies with rats have demonstrated that about 30–50% of the growth inhibitory effect of raw soybean meal (RSBM) is attributed to the presence of TI (Kakade et al., 1973). Trypsin inhibitors have also been reported to cause growth depression, and reduced feed efficiency and survival of some fish species (Smith, 1977; Abel et al., 1980; Viola et al., 1983; Wilson and Poe, 1985; Balogun and Ologhobo, 1989).

Heat treatment applied during commercial processing of SBM inactivates most of the TI and other heat-sensitive anti-nutritional factors (Liener, 1980). Heat treatment should be kept to a minimum due to its cost and the possibility of destroying essential amino acids, such as lysine and methionine, and reducing the availability of other nutrients (Kratzer et al., 1990; Van der Poel et al., 1995; Qin et al., 1998). Therefore, SBM heat processing must be a compromise between inactivation of anti-nutritional factors and destruction of essential nutrients.

In terrestrial animals, dietary TI are known to interfere with the proper function of trypsin and chymotrypsin leading to growth inhibition, reduction of feed efficiency, intestinal and pancreatic hypertrophy and hyperplasia (Liener, 1980). Fish species appear to be more sensitive to dietary TI than terrestrial animals (Krogdhal and Holm, 1983; Dimes et al., 1994). Dietary incorporation of SBM not only affects the fish's nutritional status but also their physiological and immunological integrity. It has been shown that dietary inclusion of commercial SBM resulted in pathological changes in the intestinal mucosa of Atlantic salmon (Van der Ingh et al., 1991; Baeverfjord and Krogdahl, 1996),

rainbow trout (Rumsey et al., 1994; Bureau et al., 1998) and chinook salmon, and the elevation of immunological parameters of rainbow trout (Rumsey et al., 1994). Thus, this study was undertaken to evaluate the effect of heat treatment on nutritional value of defatted RSBM with regard to protein denaturation and inactivation of TI on the growth performance, feed utilization, hematology, immune response and resistance of channel catfish to *Edwardsiella ictaluri*.

2. Materials and methods

2.1. Experimental diets, fish and feeding

Six practical-type diets were formulated to be isocaloric (2.8 kcal/kg diet) and isonitrogenous (34% crude protein). A diet containing 45% of commercial SBM (CSBM) served as the control. RSBM (Cargill Protein Products, Cedar Rapids, IA[2]), non-heat treated (RSBM0) and heated in an autoclave using the dry cycle at 130 °C and 22 psi for 5 (RSBM5), 10 (RSBM10), 20 (RSBM20), and 40 min (RSBM40), was used to isonitrogenously replace the CSBM in the control diet. All the ingredients were finely ground, mixed in a Hobart mixer and pelleted through a 2.4-mm diameter die in a Hobart meat grinder. The pellets were air dried at room temperature, broken into small pieces and stored in a freezer until used. Ingredients and proximate composition of the experimental diets are presented in Table 1.

USDA-ARS strain 103 channel catfish (*Ictalurus punctatus*) fingerlings (initial average body weight of 4.98 ± 0.09 g) were randomly selected and stocked into 110-l aquaria (50 fish/aquarium). The aquaria were supplied with a flow-though dechlorinated, heated city water at an initial rate of about 0.5–0.6 l/min and increased gradually to about 1 l/min by the end of the study. Water was continuously aerated using air stones. During the trial, water temperature averaged 25.4 ± 0.1 °C and dissolved oxygen averaged 6.2 ± 0.6 mg/l. Photoperiod was maintained on a 12:12-h light/dark schedule.

Fish in randomly assigned triplicate aquaria were fed one of the six experimental diets to apparent satiation twice daily for 10 weeks. The amount of feed consumed was recorded daily. All the aquaria were cleaned thoroughly and about two-thirds of water was drained once a week. On cleaning days, fish received only the afternoon meal. Fish from all aquaria were group-weighed and counted at weeks 4, 8 and 10 after 1 day of feed deprivation for growth measurement. When fish were removed for weighing, aquaria were cleaned thoroughly and drained. No feeding was done on sampling days.

At the beginning of the trial, 20 fish from the initial stock and at the end of the trial 5 fish from each aquarium were randomly sampled and pooled for whole-body composition analysis. Wet weight, liver and viscera weights were recorded for determination of hepatosomatic and visceral indices.

[2] Use of trade or manufacture's name does not imply endorsement.

Table 1
Composition and proximate analysis of the experimental diets

Ingredient	Percent in diet					
	CSBM	RSBM0	RSBM5	RSBM10	RSBM20	RSBM40
Menhaden fish meal	8.0	8.0	8.0	8.0	8.0	8.0
Commercial SBM	45.0	–	–	–	–	–
Non-heated RSBM	–	45.0	–	–	–	–
RSBM heated for 5 min	–	–	45.0	–	–	–
RSBM heated for 10 min	–	–	–	45.0	–	–
RSBM heated for 20 min	–	–	–	–	45.0	–
RSBM heated for 40 min	–	–	–	–	–	45.0
Constant components[1]	47.0	47.0	47.0	47.0	47.0	47.0
Proximate analyses (% dry matter)						
Moisture	9.46	9.85	10.69	10.72	9.98	9.75
Crude protein	33.85	34.46	34.48	34.41	34.82	34.91
Crude fat	6.48	5.98	6.11	6.05	6.45	6.21
Ash	7.72	7.83	7.82	7.87	7.90	7.86

[1]Constant components (% of diet): corn meal, 34.0; wheat middlings, 5.0; cod liver oil, 3.0; dicalcium phosphate, 1.0; trace mineral mix[2], 0.5; vitamin mix[3], 0.5; carboxymethyl cellulose, 3.0.
[2]Trace mineral mix provided the following minerals (mg/kg diet): zinc (as $ZnSO_4 \cdot 7H_2O$), 150; iron (as $FeSO_4 \cdot 7H_2O$), 40; manganese (as $MnSO_4 \cdot H_2O$), 25; copper (as $CuCl_2$), 3; iodine (as KI), 5; cobalt (as $CoCl_2 \cdot 6H_2O$), 0.05; selenium (as Na_2SeO_3), 0.09.
[3]Vitamin mix provided the following vitamins (mg/kg diet unless otherwise stated): vitamin A, 4000 IU; vitamin D_3, 2000 IU; vitamin K, 10; vitamin E, 50; thiamin, 10; riboflavin, 12; pyridoxine, 10; panthothenic acid, 32; nicotinic acid, 80; folic acid, 2; biotin, 0.2; vitamin B_{12}, 0.01; choline chloride, 400; L-ascorbyl-2-polyphosphate (15% vitamin C activity), 60.

2.2. Hematological and immunological assays

At the end of the trial, 16 h after the last feeding, five fish per aquarium were randomly chosen and anesthetized with tricaine methanesulfonate (MS-222) at 150 mg/l. Blood samples were collected from the caudal vein, immediately centrifuged and the plasma frozen at −80 °C for measurements of total protein content, lysozyme and pre-challenge agglutinating antibody titer against *E. ictaluri*. Plasma protein content was measured using a protein kit (BCA Protein Assay Kit, Pierce, Rockford, IL). Plasma lysozyme activity was measured by the modified method of Parry et al. (1965), using a turbidity assay in which 0.25-mg/l lyophilized *Micrococos lysodekticus* (Sigma, St. Louis, MO) in 0.04-M acetate buffer (pH 5.5) was used as the substrate.

Another five fish per aquarium were randomly sampled, anesthetized and bled for determination of hematocrit, hemoglobin and cell counts (total and red blood cells). Hematocrit value was determined by the microhematocrit method (Brown, 1988). Total cell and red cell counts were determined by diluting whole blood and enumeration in a hemacytometer. Hemoglobin was determined by the total hemoglobin kit, (Sigma Diagnostics, Sigma) a standardized procedure of the cyanomethemoglobin method. The hemoglobin values were adjusted by the cyanomethemoglobin correction factor for channel catfish as described by Larsen (1964).

2.3. Chemotaxis assay

At the end of the growth trial, four fish from each aquarium were randomly chosen and transferred into 57-l aquaria and continued to be fed their respective experimental diets. Fish were allowed to adapt to the new experimental conditions for 1 week.

Collection and isolation of peritoneal exudate cells followed the procedure of Klesius and Sealey (1996). Cells were attracted to the peritoneal cavity of fish by injecting intraperitoneally with 0.25 ml of squalene (Sigma). Five to seven days later, fish were anesthetized with MS-222 and injected (IP) with 10-ml cold, sterile phosphate-buffed saline (PBS) solution. PBS was then removed along with the squalene-elicited exudate cells into a 50-ml centrifuge tube using a 20-gauge needle attached to a three-way valve. The peritoneal fluid from four fish from the same aquarium were combined and centrifuged at $300 \times g$ for 10 min. The supernatant was discarded and the cells suspended in calcium- and magnesium-free Hank's Balance Salt Solution (HBSS) without phenol red (Gibco, Grand Island, NY) for chemotaxis assay. Cells counts and viability were established following enumeration with a hemacytometer in 0.4% Trypan Blue counting solution.

Chemotaxis was determined by a modification of the lower-surface method of Boyden (1962) as described by Klesius and Sealey (1996). Assays were performed in triplicate using blind well chemotactic chambers (Corning CoStar, Cambridge, MA) and 8-μm pore diameter polycarbonate membrane filters (Nucleopore, Pleasonton, CA) pre-soaked for 5 min in RPMI-1640 (Gilco) containing 1% horse serum. In the bottom of each chamber, 20-μl of *E. ictaluri* exoantigen (6.10 mg protein/ml) (Klesius, 1993) was added together with RPMI-1640 containing 1% of horse serum. Peritoneal macrophages were added to the upper compartment of the chamber at a concentration of approximately 5×10^5 cells/chamber. The chambers were incubated on a horizontal platform shaker (100 rpm) for 90 min at room temperature. Following incubation, filters were removed, inverted, placed on a slide, attached with clear finger nail polish and stained with Leukostat. The number of macrophages on the surface of the filter was counted in five fields of triplicate filters at 100 ×.

2.4. E. ictaluri challenge

E. ictaluri (AL-75–94) from a virulent outbreak of enteric septicemia of catfish was used for infection by bath immersion (Klesius, 1992). Stock culture of *E. ictaluri* frozen at − 80 °C (1.0-ml) was inoculated in 250-ml of brain–heart infusion (BHI) broth and cultured for 24 h at 25 °C. The concentration of the culture was adjusted to an optical density of 1.7–1.8 at 540 nm, using a spectrophotometer, to give an *E. ictaluri* concentration of approximately 1×10^9 colony forming units/ml.

To determine the optimum bacterial concentration to use in the challenge study, six groups of 20 fish, which were held in separate aquaria and fed the CSBM diet for 8 weeks, were placed in 10-l perforated plastic buckets and immersed for 30 min in static, aerated 18.4-l plastic buckets containing 10-l of *E. ictaluri* suspension at concentrations of 0; 2×10^6; 4×10^6; 8×10^6; 1.6×10^7; 3.2×10^7 cells/ml. Fish were then transferred to a new, randomly assigned 57-l aquarium. Water flow and feeding were discontinued for the first 24 h after challenge and mortality was record twice daily for 15 days. The LC_{50} (concentration lethal to 50% of exposed fish), calculated by the Karber method (Plumb

and Bowser, 1983), was 2×10^6 cells/ml and was the concentration used for the experimental challenge.

At the end of week 10, 25 fish from each replicate tank were randomly selected, placed in a perforated plastic bucket and challenged by immersion for 30 min in aerated static water containing 2×10^6 cells/ml of *E. ictaluri*. After challenge, each group of fish was removed and randomly assigned to a 57-l aquarium. Water temperature was maintained at 25 ± 2 °C. Water flow and feeding were discontinued for the first 24 h after challenge Then flow rate was established (0.5–0.6 l/min) and each group was fed twice daily with the same experimental diet that was assigned in the growth trial. Mortality was recorded twice daily for 14 days.

2.5. Agglutinating antibody titer assay

Fifteen days after *E. ictaluri* challenge, four surviving fish per aquarium were randomly chosen, anesthetized with MS-222 and bled. Blood samples were collected from the caudal vein, immediately centrifuged at $1000 \times g$ for 5 min and plasma stored frozen at -80 °C for subsequent determination of post-challenge agglutinating antibody titer against *E. ictaluri*.

Agglutinating antibody titer to *E. ictaluri* (AL-75–94) in pre- and post-challenge plasma was determined by modifying the method of Chen and Light (1994). *E. ictaluri* were grown in BHI broth for 24 h at 25 °C and killed in 1% formalin. The cells were centrifuged at $3000 \times g$ for 15 min. The resulting cell pellet was washed three times in 0.85% saline solution and suspended in sterile PBS to an optical density of 0.8 at 540 nm. Each well of a 96 round-bottom microtiter plates was plated with 15 μl of sterile PBS and then 15 μl of plasma was added to the first well of each row and mixed. Two-fold serial dilutions were then made by adding 15 μl of diluted plasma onto the remaining wells. An equal volume (15 μl) of bacterial suspension was then added to each well. Thus, the initial diluting of the plasma was 1:4. Positive (plasma from an *E. ictaluri* infected fish) and negative plasma were used as assay controls. The plates were covered with plastic film and incubated at room temperature for 16 h. The agglutination end point was established at the last dilution where cell agglutination was visible.

2.6. Digestibility

Juvenile channel catfish (USDA-ARS strain 103), averaging 22 ± 0.54 g of body weight, were randomly stocked into 12, 110-l aquarium at a density of 22 fish per aquarium. Aquaria were supplied with a flow-though dechlorinated city water at a rate of about 1.0 l/min. The fish were allowed to adapt to the experimental conditions for 15 days. During this period, they were fed with the control diet (CSBM diet) used in the growth trial. Thereafter, duplicate groups of fish were fed one of the six experimental diets containing 1% of chromium oxide to apparent satiation once daily for 10 days prior to collection of feces. Feces were collected from the rectum 14 h after feeding. To minimize the loss of feces during capture and handling, fish were killed in the aquarium with an over dose of MS-222. They were then removed and chilled with crushed ice to minimize

Table 2
Trypsin inhibitor (TI) content and protein dispersibility index (PDI) of commercial soybean meal and defatted raw soybean meal heated at various time periods

Ingredient	Heating duration[1] (min)	TI (mg/g sample)[2] (as-is basis)	PDI[3] (%)
Commercial SBM	–	4.00	23.8
Defatted RSBM	0	38.96	87.0
	5	35.00	79.1
	10	40.26	72.1
	20	19.21	40.1
	40	<1.05	6.5

[1] Defatted raw soybean meal heated in an autoclave using the dry cycle at 130 °C and 22 psi.
[2] One trypsin unit (1 TU or 1 TIU) is arbitrarily defined as an increase of 0.01 absorbance units at 410 nm, under the experimental conditions. Since 1 μg of pure trypsin increases absorbance 1.9 TU, then 1.9 TIU is equivalent to 1 μg of trypsin inhibited.
[3] Protein dispersity index.

digestive enzyme activity. Fish were dissected and fecal matter in the rectal region was collected. The rectum was designated as the area between the anus and the sphincter-like structure approximately 10–15 cm proximal to the anus. Feces of fish from the same aquarium were pooled and dried in an oven at 65 °C to constant weight. Fecal samples were ground with a mortar and pestle and stored in a dessicator for subsequent chemical analysis.

2.7. Chemical analysis

TI and protein dispersibility index of commercial, raw and heat-treated RSBM were determined using the methods described by AOCS (American Oil Chemists' Society) (1983). Chemical analyses of the experimental diets, whole fish and feces were performed using the following procedures: dry matter after drying in an oven at 105 °C until constant weight; ash by nitric acid digestion followed by incineration in a muffle furnace at 600 °C for 3 h; protein (N × 6.25) by combustion method of AOAC (Association of Official Analytical Chemists) (1998) using LECO FP-2000 analyzer; lipid by petroleum ether extraction in a Soxtec System HT apparatus; chromic oxide of feces and diets by acid digestion according to Furukawa and Tsukahara (1966).

Table 3
Apparent dry matter and protein digestibility coefficients of the experimental diets (%)

Diets	Dry matter	Protein
CSBM	75.89[b]	89.57[d]
RSBM0	71.48[a]	71.12[a]
RSBM5	71.30[a]	72.43[ab]
RSBM10	71.60[a]	72.23[ab]
RSBM20	72.46[a]	73.87[b]
RSBM40	75.66[b]	85.53[c]
Pooled SEM	0.37	0.52

Column means having the same superscript are not significantly different (P>0.05).

Table 4
Mean weight gain, dry matter feed intake (FI), feed efficiency ratio (FCR), protein efficiency ratio (PER), apparent protein utilization (APU) and survival of channel catfish fed various experimental diets for 10 weeks[1]

Diets	Weight gain (g/fish)	FI (g DM/fish)	FER[2]	PER[3]	APU[4] (%)	Survival (%)
CSBM	31.8[c]	43.5[d]	0.73[d]	2.17[d]	32.49[c]	100.00
RSBM0	14.7[a]	25.5[ab]	0.58[bc]	1.67[bc]	24.33[ab]	96.00
RSBM5	12.1[a]	23.8[a]	0.51[a]	1.48[a]	21.20[a]	99.35
RSBM10	13.3[a]	24.6[a]	0.54[ab]	1.57[ab]	22.52[a]	99.35
RSBM20	18.4[b]	29.5[b]	0.62[c]	1.79[c]	26.24[b]	100.00
RSBM40	30.9[c]	38.7[c]	0.80[e]	2.28[d]	35.23[c]	100.00
Pooled SME	1.2	1.5	0.02	0.05	1.04	0.90

[1] Column means having the same superscript are not significantly different ($P>0.05$).
[2] Feed efficiency ratio = (wet weight gain/dry feed fed).
[3] Protein efficiency ratio = (wet weight gain/crude protein fed).
[4] Apparent protein utilization = (body protein gain/crude protein fed).

2.8. Statistical analysis

Statistical analysis of the data was done by one-way analysis of variance (ANOVA) using the general linear procedure of SAS (SAS Institute, 1993). Significant differences among means ($P<0.05$) were determined by Duncan's multiple range test.

3. Results

Heating RSBM for 20 min or longer lowered trypsin inhibitor (TI) content and the RSBM40 had the lowest TI value (Table 2). The protein dispersibility index (PDI) decreased with an increase in the duration of heat treatment.

Table 5
Whole-body proximate composition (% wet weight), hepatosomatic and visceral indices (HSI and VI) of channel catfish fed various experimental diets for 10 weeks[1]

Diets	Dry matter	Crude protein	Lipids	Ash	HSI[2]	VI[3]
CSBM	71.53	14.69[bc]	10.55	1.90	1.08[a]	6.79[a]
RSBM0	72.17	14.10[ab]	10.18	1.78	1.29[b]	8.24[b]
RSBM5	73.05	13.91[a]	9.58	1.80	1.26[b]	8.08[b]
RSBM10	72.90	13.92[a]	9.36	2.00	1.32[b]	8.42[b]
RSBM20	71.78	14.14[ab]	10.8	1.88	1.28[b]	8.18[b]
RSBM40	72.19	14.88[c]	9.72	2.29	1.08[a]	6.64[a]
Pooled SME	0.45	0.21	0.40	0.12	0.05	0.36

[1] Column means having the same superscript are not significantly different ($P>0.05$).
[2] Hepatosomatic index = (liver weight/body weight) × 100.
[3] Visceral index = (visceral weight/body weight) × 100.

Table 6
Mean total cell count (TCC), red blood cell count (RBC), hematocrit, hemoglobin, and plasma lysozyme and protein content of channel catfish fed various experimental diets for 10 weeks[1]

Diet	TCC (10^6/μl)	RBC (10^6/μl)	Hematocrit (%)	Hemoglobin (g/dl)	Plasma	
					Lysozyme (μg/ml)	Protein (mg/ml)
CSBM	2.82	2.41	30.88	8.84	37.23[b]	33.26[b]
RSBM0	2.65	2.10	32.88	8.08	29.65[a]	29.32[a]
RSBM5	2.40	2.05	32.00	6.99	29.14[a]	30.85[a]
RSBM10	2.70	2.34	30.51	7.58	30.24[a]	29.59[a]
RSBM20	2.64	2.14	31.38	8.92	28.49[a]	30.68[a]
RSBM40	2.69	2.35	34.13	8.83	38.24[b]	33.93[b]
Pooled SME	0.16	0.15	1.40	0.54	1.63	0.61

[1] Column means having the same superscript are not significantly different ($P>0.05$).

Heating RSBM up to 20 min had no effect on apparent dry matter digestibility (ADMD). However, heating RSBM for 40 min improved the ADMD to a value similar to that of the CSBM (Table 3). The apparent protein digestibility (APD) significantly increased when RSBM was heated for 20 min or longer with RSBM40 having the highest APD. This value, however, was significantly lower than that of the CSBM.

Mean final weight gain, feed intake, feed efficiency ratio, protein efficiency ratio, apparent protein utilization and survival are presented in Table 4. Fish fed CSBM and RSBM40 diets had similar weight gain, protein efficiency ratio and apparent protein utilization which were significantly higher than those of fish fed the other diets. Feed intake significantly increased when RSBM was heated for 20 min or longer. Fish fed the RSBM40 diet had significantly lower feed intake but higher feed efficiency than those of fish fed the CSBM diet. The survival was not affected by dietary treatments.

Whole body composition was unaffected by dietary treatments except for the protein content of fish fed the RSBM40 diet which was significantly higher than those of fish fed

Table 7
Mean macrophage migration and chemotaxis index of channel catfish fed various experimental diets for 10 weeks[1]

Diet	Mean macrophage migration		Macrophage chemotaxis index[2]
	Control (0 μg exoantigen)	Exoantigen (120 μg exoantigen)	
CSBM	2.78	4.69[a]	1.76[a]
RSBM0	2.65	12.62[c]	4.80[c]
RSBM5	2.46	5.11[a]	2.10[a]
RSBM10	2.63	5.67[ab]	2.21[ab]
RSBM20	2.47	7.17[b]	2.91[b]
RSBM40	2.60	4.93[a]	1.90[a]
Pooled MSE	0.19	0.55	0.28

[1] Column means having the same superscript are not significantly different ($P>0.05$).

[2] Macrophage chemotaxis index was determined by dividing mean number of macrophages migrating in the presence of exoantigen by mean number of macrophages migrating in the absence of exoantigen.

Table 8
Mean days of first mortality, cumulative mortality and agglutinating antibody titer against *E. ictaluri* of channel catfish at 14 post-immersion challenge with *E. ictaluri*[1]

Diet	Days to first mortality	Cumulative mortality (%)	Antibody titer (log_{10})
CSBM	3.33	86.67[c]	2.77[a]
RSBM0	3.67	36.00[ab]	3.48[c]
RSBM5	3.67	22.67[a]	3.14[b]
RSBM10	2.67	17.33[a]	3.12[b]
SBM20	4.33	42.66[b]	3.08[b]
RSBM40	3.00	68.00[bc]	2.87[ab]
Pooled SME	0.61	10.55	0.09

[1] Column means having the same superscript are not significantly different ($P>0.05$).

the other RSBM containing diets (Table 5). There were no significant differences among the protein content of fish fed the RSBM40 and CSBM diets. Hepatosomatic and visceral indices (HSI and VI) of the groups fed CSBM and RSBM40 diets were significantly lower than those fed other diets.

Total cell count, red blood cell count, hematocrit and hemoglobin were not significantly affected by dietary treatment (Table 6). Plasma lysozyme activity and protein of fish fed the CSBM and RSBM40 diets were similar and significantly higher than those of fish fed the other diets. No significant differences were observed among plasma lysozyme and protein of fish fed the RSBM0, RSBM5, RSBM10 and RSBM20 diets.

Mean macrophage migration in the absence or presence of exoantigen and macrophage chemotaxis index are presented in Table 7. Macrophage migration in the absence of exoantigen was not significantly affected by the dietary treatment. Macrophage chemotaxis in the presence of exoantigen was higher for the groups fed the RSBM containing diets than those fed the CSBM diet, although the differences were not always significant. A similar trend was observed when the values were expressed in terms of macrophage chemotaxis index.

Cumulative mortality 14-day post challenge with *E. ictaluri* was significantly lower for fish fed the RSMB5 and RSBM10 diets but significantly increased when heating time was increased to 20 min or higher (Table 8). Fish fed the CSBM diet had the highest mortality but was not significantly different from that of fish fed the RSBM40 diet. Post-challenge antibody titer against *E. ictaluri* was significantly lower for the group fed the CSBM diet than those fed the RSBM containing diets, except for that of fish fed the RSBM40 diet. Significantly highest antibody titer was observed in fish fed the RSBM0 diet. There were no differences among the values for fish fed diets containing RSBM heated from 5 to 40 min.

4. Discussion

RSBM contains a number of anti-nutritional factors that must be removed or inactivated before it is suitable for use in fish feeds. The most commonly known and studied anti-nutritional factors are the inhibitors of protease enzymes, trypsin and chymotrypsin. These

inhibitors known as trypsin inhibitors (TI) can be destroyed by proper heat treatment (Grant, 1989). Heated SBM products need to be evaluated for the adequacy of the heat treatment to effectively destroy the anti-nutritional factors without affecting their nutritional quality. Both, chemical indicators (such as trypsin inhibitor content, protein solubility or dispersity index and urease activity) and biological indicators (such as growth, feed utilization efficiency, digestibility and gross or subclinical signs) have been used to determine the adequacy of heat treatments (Lim and Akiyama, 1991). SBM products with a TI content of 1–5 mg/g and a protein solubility index of 60–80% are considered suitable for the majority of aquaculture species namely common carp (*Cyprinus carpio*; Viola et al., 1983), channel catfish (Wilson and Poe, 1985), rainbow trout (Olli and Krogdahl, 1994) and Atlantic salmon (Olli and Krogdahl, 1994; Olli et al., 1994a). Earlier research has also shown that increasing the moisture content of RSBM prior to heat treatment decreased the heating time necessary to inactivate TI (McNaughton and Reece, 1980; McNaughton et al., 1981; Arndt et al., 1999; White et al., 2000). In the present study, for RSBM with a moisture content of approximately 8%, 40 min of heating in an autoclave using the dry cycle at 130 °C and 22 psi was adequate to decrease the TI content from 39.0 to 1.1 mg/g, a level reported acceptable for most fish species. Based on growth, feed efficiency ratio, protein efficiency ratio, apparent protein utilization and dry matter digestibility, this level of heat treatment was also sufficient to improve the nutritional value of RSBM to a level comparable to that of CSBM. It has been demonstrated that dietary incorporation of proper-heated RSBM improved the growth performance, feed intake and feed efficiency of various teleost species (Wilson and Poe, 1985; Viola et al., 1983; Balogun and Ologhobo, 1989; Falaye and Ahwieh, 1998; Arndt et al., 1999).

Present data on apparent digestibility confirm the necessity of heat treatment of RSBM to increase dry matter and protein digestibility, as has been reported for salmonids (Smith et al., 1980; Haard et al., 1996; Arndt et al., 1999) The adverse effect of dietary incorporation of RSBM or inadequately heated RSBM on digestibility has been attributed mainly to the insoluble complexes formed by TI and proteolytic enzymes, trypsin and chymotrypsin (Rajko and Szabo, 1997). Salmonids seem to be able to compensate for a certain dietary TI level by increasing trypsin synthesis and secretion (Krogdahl et al., 1994; Olli et al., 1994b; Haard et al., 1996). However, high dietary levels of TI significantly reduced protein digestibility (Olli et al. 1994b; Haard et al., 1996). Excessive or prolonged heating can lead to a decrease in the nutritional value of SBM products, primarily as a result of losing lysine in non-enzymatic browning reactions and destruction of sulfur amino acids (Lim and Akiyama, 1991). The deleterious effect of over-heating RSBM on its nutritional value was earlier reported in teleost species (Viola et al., 1983; Wilson and Poe, 1985; Fowler, 1980). In our study, although autoclaving of RSBM for 40 min improved the growth performance, feed utilization efficiency and apparent dry matter digestibility, the significantly lower dry matter feed intake, protein dispersity index and protein digestibility of the RSBM40 relative to those of the CSBM indicate that optimum heating time should be less than 40 min.

Fish fed the CSBM and RSBM40 diets had increased whole body protein content but decreased hepatosomatic and visceral indices. This agrees with previous studies in terrestrial animals that showed that muscle weight and whole-body protein content were

significantly reduced (Castell and Cliplef, 1988) whereas relative liver weight was significantly increased (Nitsan and Nir, 1977; Castell and Cliplef, 1988) when fed diets containing high levels of RSBM.

Mean total cell count, red blood cell count, hematocrit and hemoglobin did not significantly differ among treatments and these values are within the range reported for normal, healthy juvenile channel catfish (Grizzle and Rogers, 1976; Lim and Klesius, 1997; Barros et al., 2002). Thus, heat treatment of RSBM had no effect on erythropoesis. Rumsey et al. (1994) reported similar hematocrit level in rainbow trout (*Oncorhynchus mykiss*) fed diets containing low-temperature fish meal, soy protein concentrate or soybean meal. However, a higher leukocyte count was observed in fish fed diets containing soy products.

No studies have been carried out to assess the effect of heat treatment of raw soybean on immune response and disease resistance in fish. Few studies have been conducted to evaluate the effect of feeding soybean products on immune responses and disease resistance in salmonids. Krogdahl et al. (2000) observed that Atlantic salmon fed a diet containing soybean meal supplemented with an alcohol-extract of soybean meal had increased lysozyme activities and immunoglobulin in the intestinal mucosa. Rumsey et al. (1994) reported that rainbow trout fed a soybean meal diet high in heat-resistant globular proteins, glycinin and β-conglycinin, had reduced growth and impaired feed utilization efficiency but increased levels of plasma protein, immunoglobulin, production of oxidative radicals and phagocitic index. It was suggested that the increase in these immune parameters may be a result of hypersensitivity reaction or inflammatory response in the digestive tract (Rumsey et al. 1994; Krogdahl et al. 2000). Burrells et al. (1999) indicated that high concentrations of dietary soybean products suppressed the growth rate and head kidney macrophage activity in rainbow trout. The immuno-suppression became evident at dietary inclusion levels of 60–70% and was coincident with a reduction in weight gain and the appearance pathological changes in the distal intestine. They also reported no evidence of circulating antibody responses at dietary levels of soybean meal ranging from 10% to 89%. In the present study, we observed that fish fed CSBM and RSBM40 diets which exhibited better growth and feed utilization efficiency than those of the groups fed under-heated RSBM diets had significantly increased plasma lysozyme and protein levels, but decreased number of macrophage migration in the presence of exoantigen, antibody titer and increased mortality due to *E. ictaluri* infection. Although histopathological examinations of the intestinal tissues were not performed in this study, the increase in plasma protein concentration and lysozyme activity in fish fed CSBM and RSBM40 diet but not in fish fed under-heated RSBM diets may be related to other factors besides inflammatory responses.

Species differences may account for the differences between the results of these studies. Channel catfish can tolerate much higher levels of dietary soybean meal than salmonids. Soybean meal can comprise up to 50% in commercial diets of this species (NRC (National Research Council), 1993). The lower nutritional value and/or the presence of certain heat-sensitive compounds such as trypsin inhibitors, lectins and isoflavones in inadequately heated RSBM may have also contributed to the reduction in plasma protein and lysozyme activity. Moreover, one or more of these compounds may have had a stimulatory effect on macrophage chemotaxis and antibody production, and improved the resistance of channel

catfish against *E. ictaluri* infection. Rumsey et al. (1994) suggested that the stimulation of the non-specific immune mechanisms in rainbow trout fed the soybean meal diet provided the fish with some protection against infectious diseases. On the other hand, Krogdahl et al. (2000) reported that the general health of Atlantic salmon fed the soybean meal diet may have been compromised since fish in this treatment had significantly higher mortality following a 28-day challenge with *Aerominas salmonicida* as compared to those of fish fed fish meal or soy protein concentrate diets. They suggested that the damage of microvilli resulted from feeding high dietary levels of soybean meal permitted *A. salmonicida* to enter the host through the intestine, thus increased the susceptibility of fish to infection. Similar observation has been reported for *Escherichia coli* in chickens (Nitsan et al., 1989). For *E. ictaluri*, although the mechanisms involved its adhesion and penetration into host tissues are not fully understood, this was probably not the case since the olfactory organ has been suggested to be one of the primary infection sites (Wolfe et al., 1998).

Results of this study indicated that autoclaving RSBM using the dry cycle at 130 °C for 40 min lowered the TI and PDI, and improved the growth performance and feed utilization efficiency. Fish fed the RSBM40 diet had also increased body and plasma protein content, plasma lysozyme activity, and reduced HIS and VI similar to those obtained in fish fed the CSBM diet. However, based on feed intake, protein digestibility value and protein dispersity index, it appears that optimum heating time should be less than 40 min. The duration of heat treatment had no effect on hematological parameters. However, fish fed diets containing inadequately heat-treated RSBM showed an increased in macrophage migration in the presence of exoantigen, antibody titer against *E. ictaluri* and improved resistance to *E. ictaluri* infection. Raw soybean meal may contain heat-sensitive compounds or factors that improved the resistance of juvenile channel catfish against *E. ictaluri* infection. Further studies to identify these compounds and determine the mechanisms in which these compounds affect the immune system and disease resistance in fish are needed.

Acknowledgements

This research was supported by a grant (BPD/22088/99) from Fundação para a Ciência e Tecnologia, Portugal and the CRIS Project #6420-32000-011-00D of the US Department of Agriculture, Agricultural Research Service. The authors thank Rashida Eljack for the excellent technical assistance.

References

Abel, H.G., Becker, K., Meske, C.H.R., Friedrich, W., 1980. Possibilities of using heat-treated full-fat soybeans in carp feeds. Aquaculture 42, 97–108.

AOAC (Association of Official Analytical Chemists) International, 1998. Official Methods of Analysis of AOAC International, 16th ed. AOAC International, Gaithersburg, MD.

AOCS (American Oil Chemists' Society), 1983. In: Walker, R.C. (Ed.), Official Methods and Tentative Practices of the American Oil Chemists. AOCS, Champaign, IL. 840 pp.

Arndt, R.E., Hardy, R.W., Sugiura, S.H., Dong, F.M., 1999. Effects of heat treatment and substitution level on

palatability and nutritional value of soy defatted flour in feeds for Coho Salmon, *Oncorhynchus kisutch*. Aquaculture 180, 129–145.

Baeverfjord, G., Krogdahl, Å., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. J. Fish Dis. 19, 375–387.

Balogun, A.M., Ologhobo, A.D., 1989. Growth performance and nutrient utilization of fingerling *Clarias gariepinus* (Burchell) fed raw and cooked soybean diets. Aquaculture 76, 119–126.

Barros, M.M., Lim, C., Klesius, P.H., 2002. Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of channel catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri*) challenge. Aquaculture 207, 263–279.

Beckmann, J., Pfeffer, E., 1989. Hydrothermically treated fullfat soybeans in rainbow trout (*Salmo gairdneri*). In: Huisman, J., Van der Poel, T.F.B., Liener, I.E. (Eds.), Recent Advances of Research in Antinutritional Factors in Legume Seeds. Pudoc, Wageningen, pp. 99–102.

Boyden, S., 1962. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. J. Exp. Med. 115, 453–466.

Brown, B.A., 1988. Routine hematology procedures. In: Brown, B.A. (Ed.), Hematology: Principles and Procedures. Leo and Febiger, Philadelphia, PA, pp. 79–122.

Bureau, D.P., Harris, A.M., Cho, C.Y., 1998. The effects of purified alcohol extracts from soy products on feed intake and growth of Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture 161, 27–43.

Burrells, C., Williams, P.D., Southgate, P.J., Crampton, V.O., 1999. Immunological, physiological and pathological responses of rainbow trout (*Oncorhynchus mykiss*) to increasing dietary concentration of soybean proteins. Vet. Immunol. Immunopathol. 72, 277–288.

Castell, A.G., Cliplef, R.L., 1988. Performance and carcass responses to dietary inclusion of raw soybeans (cv. *Maple amber*) by boars fed ad libitum from 30 to 95 kilograms live weight. Can. J. Anim. Sci. 68, 275–282.

Chen, M.F., Light, T.S., 1994. Specificity of channel catfish antibody to *Edwardsiella ictaluri*. J. Aquat. Anim. Health 6, 266–270.

Dimes, L.E., Haard, N.F., Dong, F.M., Rasco, B.A., Forster, I.P., Farigrieve, W.T., Arndt, R., Hardy, R.W., Barrows, F.T., Higgs, D.A., 1994. Estimation of protein digestibility: II. In vitro assay of protein in salmonid feeds. Comp. Biochem. Physiol. 108A, 363–370.

Falaye, A.E., Ahwieh, V.E., 1998. The nutritive value of processed soybean hull meals in diets for the African catfish *Clarias gariepinus*. African Fishes and Fisheries Diversity and Utilisation. Poissons et Peches Africains Diversite et Utilisation, FISA/PARADI, Grahamstown (South Africa), p. 206.

Fowler, L.G., 1980. Substitution of soybean and cottonseed products for fish meal in diets fed to chinook and Coho salmon. Prog. Fish-Cult. 42, 87–91.

Furukawa, A., Tsukahara, H., 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bull. Jpn. Soc. Sci. Fish. 32, 502–506.

Grant, G., 1989. Anti-nutritional effects of soybean: a review. Prog. Food Nutr. Sci. 13, 317–348.

Grizzle, J.M., Rogers, W.A., 1976. Anatomy and Histology of Channel Catfish. Auburn University Agriculture Experimental Station, Auburn, AL.

Haard, N.F., Dimes, L.E., Arndt, R.E., Dong, F.M., 1996. Estimation of protein digestibility: IV. Digestive proteinases from the pyloric caeca of Coho salmon (*Oncorhynchus kisutch*) fed diets containing soybean meal. Comp. Biochem. Physiol. 115B, 533–540.

Kakade, M.L., Hoffa, D.E., Liener, I.E., 1973. Contribution of trypsin inhibitors to the deleterious effects of unheated soybeans fed to rats. J. Nutr. 103, 1772–1778.

Klesius, P.H., 1992. Carrier state of channel catfish infected with *Edwardsiella ictaluri*. J. Aquat. Anim. Health 4, 227–230.

Klesius, P.H., 1993. Rapid enzyme-linked immunosorbent tests for detecting antibodies to *Edwardsiella ictaluri* in channel catfish, *Ictalurus punctatus*, using exoantigen. Vet. Immunol. Immunopathol. 36, 359–368.

Klesius, P.H., Sealey, W.M., 1996. Chemotatic and chemokinetic responses of channel catfish macrophages to exoantigen from *Edwardsiella ictaluri*. J. Aquat. Anim. Health 8, 314–318.

Kratzer, F.H., Bersch, S., Vohra, P., 1990. Evaluation of heat-damage to protein by coomassie blue G dye-binding. J. Food Sci. 55, 805–807.

Krogdhal, Å., Holm, H., 1983. Pancreatic proteinases from man, trout, rat, pig, cow, mink and fox. Enzyme

activities and inhibition by soybean and lima bean proteinase inhibitors. Comp. Biochem. Physiol. 74B, 403–409.
Krogdahl, Å., Lea, T.B., Olli, J.J., 1994. Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout. Comp. Biochem. Physiol. 107, 215–217.
Krogdahl, Å., Bakke-Mckellep, A.M., Røed, K.H., Baeverfjord, G., 2000. Feeding Atlantic salmon *Salmo salar* L. soybean products: effects on disease resistance (furunculosis), and lysozyme and IgM levels in the intestinal mucosa. Aquac. Nutr. 6, 77–84.
Larsen, H.N., 1964. Comparison of various methods of hemoglobin determination of catfish blood. Prog. Fish-Cult. 25, 11–15.
Liener, I.E., 1980. Toxic Constituents of Plant Feedstuffs, 2nd ed. Academic Press, New York.
Lim, C., Akiyama, D.M., 1991. Full-fat soybean meal utilization by fish. In: Akiyama, D.M., Tan, R.K.H. (Eds.), Proc. Aquaculture Feed Processing and Nutrition Workshop, Thailand and Indonesia, pp. 188–198.
Lim, C., Dominy, W., 1989. Utilization of plant protein by warmwater fish. In: Applewhite, T.H. (Ed.), Proc. World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuff. AOCS Press, Champaign, IL, pp. 245–251.
Lim, C., Klesius, P.H., 1997. Responses of channel catfish (*Ictaluri punctatus*) fed iron-deficient and replete diets to *Edwardsiella ictaluri* challenge. Aquaculture 157, 83–93.
McNaughton, J.L., Reece, F.N., 1980. Effect of moisture content and cooking time on soybean meal urease index, trypsin inhibitor content, and broiler growth. Poultry Sci. 59, 2300–2306.
McNaughton, J.L., Reece, F.N., Deaton, J.W., 1981. Relationships between color, trypsin inhibitor contents, and urease index of soybean meal and effects on broiler performance. Poultry Sci. 60, 393–400.
Nitsan, Z., Nir, I., 1977. A comparative study of the nutritional and physiological significance of raw and heated soybeans in chicks and goslings. Br. J. Nutr. 37, 19–25.
Nitsan, Z., Dunnington, E.A., Gross, W.B., Siegel, P.B., 1989. Growth and *Escherichia coli* resistance of chickens fed diets varying in energy and soybean processing. Nutr. Rep. Int. 40, 871–877.
NRC (National Research Council), 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC. 114 pp.
Olli, J.J., Krogdahl, Å., 1994. Nutritive value of four soybean products as protein sources in diets for rainbow trout (*Oncorhynchus mykiss*, Walbaum) reared in fresh water. Acta Agric. Scand., A Anim. Sci. 44, 185–192.
Olli, J.J., Krogdahl, Å., Van den Ingh, T.S.G.A.M., Brattas, L.E., 1994a. Nutritive value of four soybean products in diets for Atlantic salmon (*Salmo salar*, L.). Acta Agric. Scand., A Anim. Sci. 44, 50–60.
Olli, J.J., Hjelmeland, K., Krogdahl, Å., 1994b. Soybean trypsin inhibitors in diets for Atlantic salmon (*Salmo salar*, L.): I. Effects on nutrient digestibilities and trypsin in pyloric caeca homogenate and intestinal content. Comp. Biochem. Physiol. 109, 923–928.
Parry, R.M., Chandan, R.C., Shahani, K.M., 1965. A rapid and sensitive assay of muramidase. Proc. Soc. Exp. Biol. Med. 199, 383–386.
Plumb, J.A., Bowser, P.R., 1983. Microbial Fish Disease Laboratory Manual. Alabama Agriculture Experiment Station, Auburn University, Auburn, AL. 95 pp.
Qin, G.X., Verstegen, M.W.A., Van der Poel, A.F.B., 1998. Effect of temperature and time during steam treatment on the protein quality of full-fat soybean from different origins. J. Sci. Food Agric. 77, 393–398.
Rajko, R., Szabo, G., 1997. Designing experiments for reducing antinutritive agents in soybean by microwave energy. J. Agric. Food Chem. 45, 3565–3569.
Rumsey, G.L., Siwicki, A.K., Anderson, D.P., Bowser, P.R., 1994. Effect of soybean protein on serological response, non-specific defense mechanisms, growth and protein utilization in rainbow trout. Vet. Immunol. Immunopathol. 41, 323–339.
Smith, R.R., 1977. Recent research involving full-fat soybean in salmonid diets. Salmonid 1, 8–18.
Smith, R.R., Peterson, M.C., Allred, A.C., 1980. Effect of leaching on apparent digestion coefficients of feed-stuffs for salmonids. Prog. Fish-Cult. 42, 195–199.
Tacon, A.G.J., 1994. Feed ingredients for carnivorous fish species: alternatives to fishmeal and other dietary resources. FAO Fish. Circ. 881, 35.
Van der Ingh, T.S.G.A.M., Krogdahl, Å., Olli, J.J., Hendriks, H.G.C.J.M., Konikx, J.G.J.F., 1991. Effects of soybean-containing diets on the proximal and distral intestine in Atlantic salmon (*Salmo salar*): a morpho-logical study. Aquaculture 94, 297–395.

Van der Poel, A.F.B., Verstegen, M.W.A., Tamminga, S., 1995. Chemical physical and nutritional effects of feed processing technology. Proc. 16th Western Nutrition Conference, Saskatoon, Canada, pp. 70–86.

Viola, S., Mokady, S., Arieli, Y., 1983. Effects of soybean processing methods on the growth of carp (*Cyprinus carpio*). Aquaculture 32, 27–38.

White, C.E., Campbell, D.R., McDowell, L.R., 2000. Effects of dry matter content on trypsin inhibitors and urease activity in heat treated soya beans fed to weaned piglets. Anim. Feed Sci. Technol. 87, 105–115.

Wilson, P., Poe, W.E., 1985. Effects of feeding soybean meal with varying trypsin inhibitor activities on growth of fingerlings channel catfish. Aquaculture 46, 19–25.

Wolfe, K.G., Plumb, J.A., Morrison, E.E., 1998. Lectin binding characteristics of the olfactory mucosa of channel catfish: potential factors in attachment of *Edwardsiella ictaluri*. J. Aquat. Anim. Health 10, 348–360.